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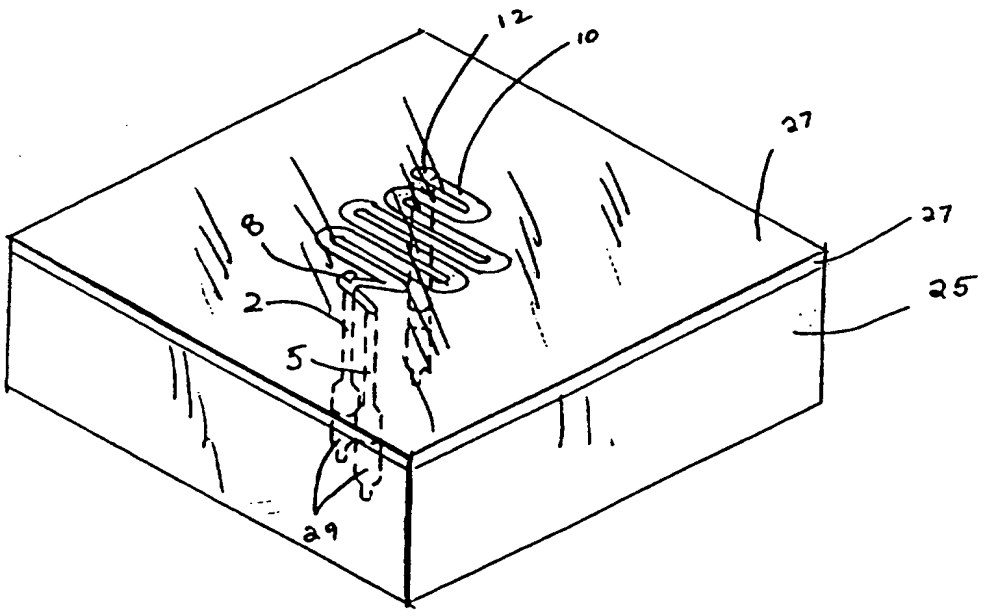
(57) Abstract

A method and article for conducting a chemiluminescent reaction in which a reaction conduit is provided having a unique configuration for enhanced chemiluminescent signal production and detection. The conduit is preferably provided with a plurality of folds defining multiple substantially different flow paths for reactants flowing therethrough. The method and article are suitable for use in diagnostic assays employing chemiluminescent labels.



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ARTICLE AND METHOD FOR CONDUCTING CHEMILUMINESCENT REACTION

Background of the Invention

The present invention relates generally to methods and articles for effectuating contact between chemical compounds which produce a detectable signal when reacted. More particularly, the invention concerns a chemiluminescence reaction cell, and a method using the same, in which a reaction conduit is specially configured to increase the amount of signal produced when a chemiluminescent reaction is carried out in the cell.

It is well known that many chemical and biochemical reactions produce light. This phenomenon, commonly known as chemiluminescence, can be used to great advantage in medical diagnostics. For example, a diagnostic test or assay which analyzes for antigens, antibodies, haptens, or other such "analytes" in human body fluids can utilize chemiluminescence by coupling a chemiluminescent compound to the analyte sought to be detected. Examples of such analytes include toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), virus particles and metabolites of or antibodies to any of the above substances. In such an assay, the chemiluminescent compound coupled with the analyte will not provide any light output until the chemiluminescent compound is "triggered" by exposure to a triggering agent. Thus, the first step in such an assay involves combining a patient sample (with or without any prior processing) with a reagent which contains the chemiluminescent compound, in the absence of trigger solution, and under conditions which will result in coupling of the chemiluminescent compound to the analyte suspected to be present in the patient sample. After such coupling, the resultant complex containing the analyte and the chemiluminescent compound is typically separated from any unbound materials in the assay and then contacted with the triggering agent in order to produce a chemiluminescent reaction. The amount of signal produced can be directly correlated to the amount of analyte present in the patient sample. Diagnostic assays of this general type, and numerous variations, are well known in the literature. Acridinium compounds such as acridinium sulfonamides and acridinium esters are examples of chemiluminescent compounds that have been disclosed for use in the type of diagnostic assay generally described above.

A problem in diagnostic assays which employ chemiluminescent detection is that it is often difficult to reproducibly obtain the greatest possible output of signal from the least amount of chemiluminescent reactant. The ability to overcome this problem is particularly important in those instances in which a diagnostic assay is required to detect very low levels of analytes and distinguish differences among such levels. Moreover, the ability to maximize signal output in a chemiluminescent assay, would facilitate a more economical use of assay reagents.

Summary of The Invention

The present invention is a method for detecting a signal produced by a chemical reaction. The method comprises the steps of (1) providing a first flowable reactant in a first conduit and, separate therefrom, providing a second flowable reactant in a second conduit, wherein the separate first and second reactants are selected such that when contacted with one another, a detectable signal is produced; (2) providing a continuous, folded detection conduit which is transparent to said detectable signal and which is connected to the first and second reactant conduits, where the detection conduit comprises at one end thereof a first opening into which the first and second reactant conduits merge so as to be able to effectuate a merged flow from said first and second separate flowable reactants, and at another end thereof a second opening for discharge of the merged flow (containing spent reactants and reaction products) out of the detection conduit, whereby the reactants present in the first and second conduits can be made to flow out of those conduits, into the detection conduit through said first opening, whereupon said merged flow can then flow out of the conduit through the second opening; the detection conduit further comprising, between the first and second openings, at least one folded section, such folded section comprising, in continuous integral interconnection (i) a first segment of the detection conduit for chanelling the merged reactants along a first flow path; (ii) a second segment of the detection conduit for channelling the merged reactants along a second flow path, such that the first and second flow paths are differently directed; and (iii) a third segment of the detection conduit constituting an elbow which joins the first and second aforementioned segments; whereby said merged flow can be channeled from said first flow path to said second flow path, and wherein said first, second and third segments together define a non-circular path of travel for said merged reactants in said folded section; (3) causing the first and second reactants to flow out of the first and second conduits and into the

detection conduit, whereupon said reactants are caused to merge with and contact one another at said first opening of the detection conduit, such that said signal is produced in said conduit; (4) providing means for detecting signal produced in step 3, such means being positioned alongside the detection conduit, and wherein the detection conduit is folded to fit its entire length or substantially its entire length within the detection field of the signal detection means; (5) using the signal detection means to measure the amount of signal produced in step 3. As used above, the term "non-circular path of travel" is intended to mean that the folded section of the detection conduit is not superimposable on the arc of a circle. Throughout this specification the terms "detection conduit" and "reaction conduit" are used interchangeably. The invention is not intended to be limited to chemical reactions in which the detected signal is in the form of light energy.

Preferably, the flow path in the abovementioned first fold segment differs substantially from the flow path of the second fold segment. As used herein, the terminology "differ substantially" with respect to describing the relationship between the flow path of the first fold segment and the flow path of the second fold segment, is intended to denote an angle between these flow paths of less than about 150°. At angles greater than this, the change in direction between the first flow path and the second flow path is less pronounced, and the benefits of the present invention become increasingly less evident as the angle approaches 180°, (at which angle there of course would be no change in flow path.) Preferably, the angle between said first and second flow paths is an acute angle, and, most preferably, a small acute angle of less than 45°. In the most prefered embodiments of the invention, the first and second fold segments are essentially linear and the first and second flow paths are disposed along lines which are essentially parallel and non-intersecting, as shown in some of the embodiments depicted in the attached figures, whereby the change in flow path in the folded detection conduit constitutes essentially a reversal in flow direction.

In a related method, the invention is further directed to a method for conducting a signal-producing chemical reaction in which the folded detection conduit described above is substituted with a spiral conduit, preferably wherein the merge point for the signal producing reactants is located at the center of the spiral such that the merged reactants react within and flow outward along a spiral path to a discharge conduit. This positioning of the reactant confluence at the center of the spiral provides for improved mixing of the merged reactants at the early stages of a chemiluminescent reaction as

compared with known methods which combine the signal producing reactants in a test tube or cuvette such as is done with commercially known luminometers.

As an article of manufacture, the invention is a reaction cell suitable for conducting a signal producing chemical reaction, comprising: a housing and three separate conduits formed in said housing; wherein the conduits merge at a single point, and wherein one of the conduits is folded so as to comprise at least one folded section comprising, in continuous integral interconnection (i) a first segment of the conduit defining a first flow path; (ii) a second segment of said conduit defining a second flow path differently directed from said first flow path; and (iii) a third segment of the folded conduit constituting an elbow joining said first and second segments, and wherein the first, second and third fold segments together define a non-circular flow path. The term "non-circular" has the same meaning as given above.

In the case of both the method and the reaction cell, the folded conduit preferably contains a plurality of folded sections where said first and second segments of each fold are essentially parallel to one another, and wherein the distance between the merge point of said three conduits, and the elbow segment nearest said merge point, is not greater than the greatest distance between any two of said elbow segments. The purpose of these preferred requirements is to provide enhanced mixing at said elbow segments, and to assure that the bulk of the signal producing reaction occurs within a field of detection after the reactants arrive at the first folded section of the folded conduit.

The method and article of the present invention provide excellent signal generation and detection for a signal-producing chemical reaction, and particularly for chemical reactions which produce a chemiluminescent signal. As a preferred embodiment, the folded reaction conduit provides an enhanced signal in comparison to a reaction cell in which the signal producing reactants are conducted along a circular reaction conduit. A further significant advantage of the invention is that the reaction cell and method can be used to perform diagnostic assays which do not require disposable assay cuvettes. Moreover, a chemiluminescent signal generated in the reaction cell of the invention is markedly enhanced over signal generated when chemiluminescent reagents are mixed in a cuvette, test-tube, or other non-folded or non-spiral mixing chamber. A further advantage of the method and article of the invention is that they make possible the performance of

remarkably sensitive and reproducible diagnostic assays using chemiluminescent labelling of analytes.

Brief Description of the Drawings

Figure 1 is a schematic representation of an S-loop reaction cell in accordance with the present invention.

Figure 2 is a perspective view of an S-loop reaction cell of the present invention.

Figure 3 is a top view of the reaction cell depicted in Figure 2.

Figure 4 is a cross section of the reaction cell of Figure 2 showing inlet ports for chemiluminscent reactants.

Figure 5 is a cross section of the reaction cell of Figure 2 showing a discharge port.

Figure 6 is a perspective view of a Z-loop reaction cell according to the present invetion.

Figure 7 is a top view of the cell depicted in Figure 6.

Figure 8 is a cross-section of the cell depicted in Figure 6 which shows two openings in the the cell, one of which 50a is for the chemiluminescent reactants, the other 48a for discharge of a merged flow out of the cell.

Figure 9 is a cross section of the cell of Figure 6 showing the reaction conduit in the cell being disposed in two separate planes and the transition of the conduit from an upper plane to a lower plane thereof.

Figure 10 is a schematic of the cell of Figure 6 depicting the dual layered nature of the reaction conduit therein.

Figure 11 is a top view of a fingerprint folded reaction cell in accordance with the present invention.

Figure 12 is a cross section of the cell of Figure 11 illustrating the angle at which the reactant entry conduits are disposed with respect to the reaction conduit.

Figure 13 is a cross section of the cell of Figure 11 illustrating a discharge conduit from said cell.

Figure 14 is a top view of a spiral flow cell according the present invention.

FIgure 15 is a cross section of the cell of Figure 14 illustrating inlet and discharge conduits.

Figure 16 is a top view of an assembly for housing a reaction cell of the present invention, including a photomultiplier tube.

Figure 17 is a cross section of the housing of figure 16.

FIgures 18-20 are schematic representations of several different folded-conduit reaction cells according to the invention.

Detailed Description

Figure 1 is a schematic representation of a chemiluminescence reaction cell according to the present invention. The reaction cell 1 is provided with three fluid-conducting conduits 2, 5 and 10 which merge at a single merge point 8. Conduits 2 and 5 are supplied fluidically with chemiluminescent reactants from sources 3, and 7 respectively. For example, source 3 may be a solution containing a chemiluminescent compound such as an acridinium compound. Specifically, in the case in which a reaction cell of the present invention is employed in a diagnostic assay, source 3 could be solution of analyte wherein the analyte has been coupled to a chemiluminescent compound such as an acridinium sulfonamide. The reactant supplied by source 7 may be a trigger solution which, when brought into contact with the chemiluminescent compound from source 3, causes production of a chemiluminescent signal (i.e., photons). Conduits 2 and 5, and the contents thereof from sources 3 and 7, are maintained separate from one another until they are merged and contacted with one another at merge point 8, thereby causing a chemiluminescent signal to be emitted as the merged flow continues into and through the reaction/detection conduit 10. The conduit 10 is a continuous single channel which extends at one end from the merge point 8 to an opposite end at discharge outlet 12 which is fluidically connected to a waste receptical 17. Between merge point 8 and discharge outlet 12 are provided a plurality of turbulence-inducing conduit folds which cause the merged flow from sources 3 and 7 to travel through conduit 10 along a tortuous or multiply reversing flow path. As seen in Figure 1, each of the conduit folds comprises a first section 13 defining a first flow path for the merged flow travelling through the conduit 10; a second section 14 defining a second flow path for the merged flow; and a third section 15 serving as an elbow section connecting the first and second flow paths. The elbow segment provides a sharp turn causing the merged flow in conduit 10 to change direction sharply when flowing from fold section 13 to fold section 14. This substantial change in direction from the first to the second flow path tends to enhance mixing at said elbow section 15, for improved mixing of the reactants in the merged flow. Although sections 13, 14 and 15 are represented using brackets it will be understood that the three sections are integrally and continuously interconnected. It should also be understood that although

sections 13, 14 and 15 are shown only one time in Figure 1, there are a total of eight folds in conduit 10 (intersections 21 and 22 may be considered folds for purposes of the invention) and each of said folds will comprise its own set of sections 13, 14 and 15. Broken line 19 represents the detection field of the chemiluminescence detection device 23. Such detection device is preferably a photomultiplier tube (PMT). The folding of detection conduit 10 provides that the conduit 10 is essentially completely within the detection field of the PMT 23. The residence time of the fluid stream in the reaction cell, and the configuration of the cell folds are such that the chemiluminescent reaction is substantially completed by the time the stream has reached the limit of the detection filed 19 and is ready to pass through discharge point 12 of conduit 10.

Figures 2 through 5 show an actual embodiment of the reaction cell depicted schematically in Figure 1. This embodiment is referred to as the "S-loop" embodiment. In Figure 2, the tortuous conduit 10 has been machined into a solid polymeric block 25 in the form of an open-topped or u-shaped channel. This machined channel is then sealed with a covering layer 27 of transparent polymeric plastic such as polycarbonate which is glued with an epoxy and/or thermally bonded onto the solid base 25. Covering layer 27 is about .15 mm in thickness and forms the top surface of conduits 2, 5 and 10. The choice of polymeric materials is not critical to manufacture of the solid block 25 and covering layer 27, except that the materials should be inert to reactants intended to be used with the cell, and top layer 27 must have excellent transparency. An example of material suitable for fabrication of solid block 25 is white opaque polyvinyl chloride. In somewhat greater detail, figures 2 through 5 illustrate a tortuous path 10 that is confined to one horizontal plane. The tortuous path 10 contains a plurality of elbows and stays within a detection zone 19 indicated by the broken circular line in Figure 3 which forms a 12 mm diameter circle. Detection zone 19 represents the viewing surface of a photomultiplier tube. Source conduits 2 and 5 are about 0.5 mm across and about 0.4 mm deep, whereas the detection conduit 10 is about 1 mm wide and about 1.2 mm deep, whereby flows merging from conduits 2 and 5 into the detection conduit 10 are subject to improved mixing. Detection conduit 10 has a volume of about 70µl. At the confluence point 8, as shown in Figure 3, a suitable trigger solution flows in from the first source conduit 2 at a 60° angle to the flow path of conduit 10, and a suitable chemiluminescent reagent flows from the second source conduit 5 into the confluence point 8 at 20° flow path angle to the detection conduit 10. The

difference in flow paths provided by the angular disposition of conduits 2 and 5 with respect to conduit 10 provide enhanced mixing and contacting of reactants at said confluence point. Fluidic connectors 29 shown in figures 2, 4 and 5 are threaded into block 25 and facilitate fluidic linkage to reactants which can be supplied from sources external to the reaction cell block 25. Figure 5 depicts the discharge conduit 12 which leads out of conduit 10 to a waste receptacle (not shown).

A further embodiment of the reaction cell of the present invention is depicted in Figures 6-10. In this embodiment referred to as the "Z-loop" embodiment, the detection conduit 35 has a plurality of zig-zag folds, and occupies two separate planes which are disposed one on top of the other. In this embodiment, as shown in figures 6 and 7, the detection conduit 35 follows a tortuous zig-zag path within a detection zone 37 forming a 9 mm diameter circle. As shown in Figures 6, 7 and 8 the detection conduit 35 repeatedly traverses the detection zone 37 on a plane near the top surface 52 of transparent polymeric block 51, to form a first or upper zig-zag network 35a of conduit folds, and then turns normal to the block surface at juncture 39 to reach a plane further below the surface 52 and below the upper zig-zag network 35a to form a second zig-zag network 35b , in which lower network of folds the discharge conduit 35 again repeatedly traverses the detection zone 37. In this embodiment the material of the block 51 should be transparent or translucent in order to allow light to travel from the lower plane to a photon detection device (not shown). The conduits are formed in block 51 using well-known plastic fabrication techniques. As shown in Figures 6 and 8, block 51 provides an annular recess for housing a circular reflecting mirror 55 which is embedded in the block. Mirror 55 corresponds in area to the detection zone 37 of the reaction cell, which in turn corresponds to the detection opening of a photomultiplier tube (not shown) which can be placed immediately above surface 52 in alignment with said detection zone 37. Mirror 55 enhances detection of light from the reaction cell by a photomultiplier tube. As shown in Figures 6, 7, 8 and 10 there are provided two separate conduits 45 and 50 for flowing chemiluminescent reactants into the detection conduit 35. Figure 8 shows the relationship of conduits 45 to the openings 50a and 48a of conduits 50 and 48. The dual-layered nature of the ziz-zag conduit, and the confluence of conduits is schematically shown in Figure 10, as well as in the cross section of Figure 9. Each of the folds in the detection conduit 35 defines two differently channeled flow paths joined by an elbow, such that the flow paths are at an angle of about 20 degrees to each other.

As can be seen in the case of the S-loop and the Z-loop embodiments, discussed above, the detection conduit is provided with a plurality of folds in which each fold defines a non-circular, flow path for enhanced mixing and detection of chemiluminescent reactants. As shown by the merge points 8 and 41 in figures 3 and 7 respectively, it is preferred that the confluence of the separate reactant conduits into the detection conduit occurs at a merge point which is within the detection zones 19 and 37 of the two embodiments in order to maximize the detection of signal output from the chemiluminescent reactants.

Still another embodiment of the reaction cell of the present invention is depicted in Figures 11-13. This embodiment is referred to as the "fingerprint" embodiment, based on the fact that the pattern of folds in the detection conduit resembles a fingerprint. The cell in figure 11 comprises a detection conduit 87 into which merge two separate conduits 80 and 82 for injecting chemiluminescent reactants into conduit 87 at merge point 85. Conduits 80 and 82 are at a 30 degree angle to each other, and are disposed at a thirty degree angle to the plane of the detection conduit 87, as shown in the cross section of Figure 12. In the detection conduit there are a plurality of folds such that the direction of flow is substantially reversed a number of times while reactants are coursed through the conduit. One of the folds of said detection conduit comprises a first section 87a, defining a first flow path, a second section 87b defining a second flow path and a third section 87c defining an elbow section which connects said first and second sections. The flow paths in the first and second sections are markedly different such that they are disposed along lines which are parallel. Like the other embodiments, the cell is formed in a solid block 70 in which an open-topped channel is formed for conduit 87, which is then covered with a transparent cover 72. The detection conduit is folded so that it is within the detection zone 90 of a photomultiplier tube.

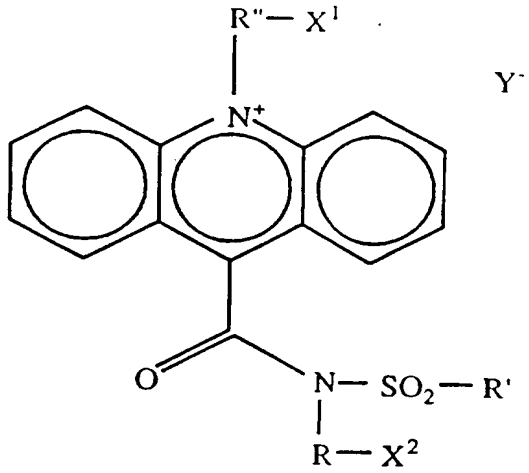
Figures 14 and 15 depict a "spiral" embodiment in which the detection conduit is in the form of a circular path. Although we have found this to be markedly less efficient for signal production in comparison to the other embodiments of the invention, the signal output is still improved over cuvette mixing and conventional luminometers. Although this embodiment does not utilize the folds of the other embodiments, it does, as shown in figures 14 and 15 utilize the feature of providing a merge point 93 for the source conduits 90 and 91 into the spiral detection conduit 94 which is within a detection zone 95. Further, it is found that enhanced mixing is achieved for chemiluminescent

acridinium compounds based on the fact that source conduits 90 and 91 are disposed at an acute angle with respect to the plane of the spiral detection conduit 94. Although folded reaction conduits are preferred for use in the present invention, a spiral detection cell nevertheless provides an excellent improvement in mixing and signal production versus test tube or cuvette mixing.

Turning now to Figures 16 and 17, there is depicted an S-loop embodiment similar to what was described earlier, except that the S-loop reaction cell is now shown as part of a detection apparatus which may be included as a subassembly in a diagnostic instrument in which chemiluminescent reactants are fluidically supplied from other portions of the apparatus (not shown) to the illustrated detection subassembly. In particular, the solid plastic S-loop detection cell 100 is mounted in a light-tight housing 120. The housing includes an extension 116 for tightly housing and securing a photomultiplier tube 106. Openings 124 in the housing are tightly sealed and permit entry of fluid conducting tubing which carries a chemiluminescent reactant in tube 104, and a trigger solution in tube 102. A fluidics delivery system (not shown) can be provided in a known manner for pumping reactants through tubing 102 and 104 into the detection loop 112. After transfer through the detection conduit 112, the spent reactant stream is discharged through tube 110 which leads to a waste receptacle. Mounting brackets 108 hold the reaction cell in place. The folded detection conduit 112 is completely within the detection zone of PMT 106. Adapters 126 are employed for connecting delivery tubing 102 and 104 to the inlet conduits of the reaction cell.

All of the embodiments of the invention described above generally operate in a manner to cause mixing of chemiluminescent reactants within a detection zone. The physical structure of the reaction conduits in each of the embodiments is such as to maximize both the amount of mixing of the two fluids and the duration of time a fluid element remains within the detection zone, thereby maximizing the signal detected by a detection device. In particular, the provision of multiple conduit folds which provide the features of abrupt and subtantial flow path changes within the detection zone; source conduits which can be narrower than the detection conduit; confluence or merge points whereby the source conduits empty into the folded detection conduit at a point which is within the detection zone, all provide for enhancement of the chemiluminescent signal.

As used herein, the term "acridinium sulfonamide" means the chemiluminescent compounds identified by the formula:



wherein R, R', R", X^1, and X^2 are substituents which do not interfere with the chemiluminescent signal provided by such chemiluminescent compounds, with the proviso that R"-X^1 and R-X^2 may be independently hydrogen. More specifically, R and R" may be spacer arms and X^1 and X^2 may be independently members selected from the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidyloxycarbonyl and N-succinimidyloxysulfonyl. Y^- is an appropriate counterion, and may be selected from the group consisting of sulfate, alkylsulfate, halosulfate, haloborate, haloacetate, halophosphate, phosphate and halide. Preferably, the counterion is sulfate or halide.

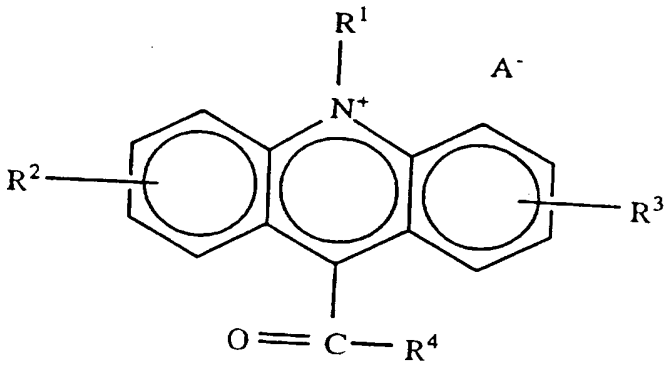
R, R', and R" may independently include a member selected from the group consisting of alkyl, alkylene, aryl, substituted alkyl, substituted alkylene, and substituted aryl groups, such that one or more hydrogens of said member can be replaced by an alkyl, aryl, alkylene, substituted alkyl, substituted alkylene, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group; or such that one or more carbon atoms of the member can be replaced by a heteroatom. The heteroatom may be selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen.

R and R" independently may also be spacer arms of the formula $-(CH_2)_n-$ where n = 0-50.

The preferred acridinium sulfonamide compounds for use in the present invention are 10-methyl-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide

and 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide. The most preferred acridinium sulfonamide is 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide.

Also suitable for use in the present invention are the acridinium sulfonamide compounds referenced in Molz et al. European Patent Application No. 257,541 (published March 2, 1988) incorporated herein by reference. The acridinium sulfonamide compounds discussed in Molz et al. have the following general formula:



In which R^1 stands for hydrogen, an alkyl, alkenyl or alkynyl radical with 1 to 10 carbon atoms, a benzyl or aryl group, R^2 and R^3 stand for hydrogen, an alkyl group with 1 to 4 carbon atoms, a substituted or unsubstituted amino group, a carboxy, alkoxy, cyano, nitro group or halogen, R^4 represents a radical in which a sulfonamide group is bound directly to the carbonyl group via the nitrogen or a thioalkyl or thioaryl radical of formula:

$$-S \bullet X \bullet R^5$$

where X is a branched or unbranched aliphatic or aromatic group which may also contain heteroatoms, and R^5 is a reactive group which selectively under gentle conditions can enter into a bond with amino, carboxy thiol or other functional groups in substances of biological interest, and A^- is an anion which does not impair chemiluminescence.

Preparation of the acridinium sulfonamide compounds useful in the present invention is disclosed in Mattingly et al. European Patent Application 273,115 published July 6, 1988, incorporated herein by reference. Molz et al. published European Patent Application No. 257,541 also discusses preparation of acridinium sulfonamides.

The acridinium sulfonamide can be oxidized by any oxidant which reacts with the acridinium sulfonamide to yield a product in an electronically excited state. As it returns to the ground state, this product releases energy in the form of light in a chemiluminescent reaction.

As used herein, a "trigger solution" means the solution containing the oxidant which initiates or catalyzes the chemiluminescent reaction. A preferred trigger solution comprises hydrogen peroxide in dilute alkali.

In addition to acridinium sulfonamides, the present invention can also be practiced using acridinium esters which are well known chemiluminescent compounds. Another chemiluminescent label which can be used in a diagnostic assay which employs the reaction cell and method of the present invention is luminol (or derivatives of luminol) using as the trigger an alkaline peroxide. Frequently a catalyst such as microperoxidase is added to the alkaline peroxide to enhance the signal output.

Enzymes can be labels which catalyze chemiluminescence when mixed with the appropriate substrate. One enzyme stubstrate pair is horseradish peroxidase with a mixture of luminol and peroxide as substrate. Enhancers such as phenolic compounds can be added to the enzyme label to generate even more light. Another widely used enzyme is alkaline phosphatase with chemiluminescent 1,2-dioxetane phosphates such as spiro-adamantyl meta-phenyl phosphate 1,2-dioxetane. Substituents on the phenyl ring can be varied to allow more rapid emission of light compatible with the rapid mixing device of the present invention.

Electrochemiluminescent reactions can be detected in the flow cell of the present invention by merging an electrochemically-generated radical cation in one flowing stream with a Ruthenium tris-bipyridyl label in the other flowing stream.

The following examples are provided to illustrate the method of the present invention.

EXAMPLE 1

An example of use of an embodiment of the present invention to demonstrate the dynamic range of chemiluminescent detection uses the S-loop embodiment shown in Figure 2. 50 µl of magnetic microparticles, a known fraction of which are bound to a chemiluminescent substance, were injected into the detection cell of Figure 2. The magnetic microparticles were Nippon Paint 006 particles (Nippon Paint Co., Tokyo, Japan) of about 1.2 µm diameter and 50 percent magnetite by weight. They were bound to sulfopropyl

acridinium sulfonamide compound (a known chemiluminescent compound) and suspended in buffer at four different concentrations (by weight) of 0, 0.00075, 0.0075 and 0.15 percent solids. The trigger substance used was an aqueous solution with 0.4% H_2O_2, 0.225 M NaOH, 2% Triton X-100, and 0.01% diethylene triamine pentaacetic acid. 50 µl of the acridinium labelled magnetic microparticle suspension were fluidically transferred to conduit 5 of the detection cell shown in Figures 2 through 5 at a rate of about 4 ml/minute. Simultaneously, trigger solution was transferred into conduit 2 of the detection cell at a rate of about 6 ml/minute. Light emission from the detection cell was measured by a Hamamatsu photomultiplier tube (Model R647-04). The light emitted was approximately proportional to the amount of chemiluminescent particles in the microparticle suspension. The following table contains the results of signal detection using the four different acridinium concentrations given above.

TABLE 1

Amount of chemiluminescent particles (% by weight)	0	0.00075	0.0075	0.15
Light emission (Relative Light Units)	1,394	8,515	71,131	1,443,194
Coefficient of Variation of light emission	5.8	1.9	0.59	1.2
Number of trials	4	4	4	4

EXAMPLE 2

This is an example of use of an embodiment of the present invention in the performance of an immunological assay using the reaction cell of figures 2 through 5. The magnetic microparticles are Nippon Paint 006 particles (Nippon Paint Co., Tokyo, Japan) of about 1.2 µm diameter and 50 percent magnetite by weight. The particles were bound to mouse anti-beta TSH (thyroid stimulating hormone) antibodies and suspended in buffer at a concentration (by weight) of 0.15 percent solids. A conjugate reagent was prepared consisting of sulfopropyl acridinium sulfonamide labeled to goat anti-alpha-hCG (human chorionic gonadotropin) antibody at a concentration of 32 ng/ml in a diluent of an aqueous solution of 10 mM MES (2-[N-Morpholino] ethanesulfonic acid), 150 mM NaCl, 2% bovine serum albumin, 0.5% Triton X-100, and 0.1% NaN_3, at a pH of about 6.3. The trigger substance was an aqueous solution with 0.4% H_2O_2, 0.225 M NaOH, 2% Triton X-100, and 0.01% diethylene triamine pentaacetic acid. The samples analyzed were

IMx® Ultrasensitive hTSH (human thyroid stimulating hormone) In Vitro Test Calibrators, catalog #3A62-01 obtained form Abbott Laboratories, Abbott Park, IL. 58 µl of microparticles and 200 µL sample (the TSH calibrators) were combined in a suitable reaction chamber and incubated for 10 minutes at room temperature (about 22°C) to form a "sandwich" complex. The magnetic microparticles were washed with buffer to remove unbound sample. The buffer is catalog #8374 from Abbott Laboratories, Abbott Park, IL, with 0.015% Brij® detergent (Sigma Chemicals, St. Louis, MO) added. 280 µl of the conjugate reagent were then added to the incubated sample and microparticles and the mixture was incubated for 7.5 minutes at room temperature (about 22°C). The microparticle containing incubation mixture was then washed twice to remove unbund conjugate. The resultant assay mixture is a suspension of microparticles containing a sandwich complex including the acridinium label. The magnetic microparticle suspension was then transferred to the first source conduit 5 of the detection cell 25 depicted in Figure 2 at a rate of about 4 ml/minute. Simultaneously, trigger is transferred into the second source conduit 2 of the detection cell at a rate of about 6 ml/minute. The flows from conduits 5 and 2 then merged into the detection conduit 10 of the reaction cell and produced a chemiluminescent signal. Light emission from the detection cell was measured by a Hamamatsu photomultiplier tube (Model R647-04). The light emitted is specifically related to the concentration of TSH in the analyzed sample. The results are reported in Table 2, below.

TABLE 2

Sample TSH concentration (µlU/ml)	0.0	0.5	2.0	10.0	40.0	100.0
Light emmission (relative light units	29,088	61,863	168,029	607,386	1,772,729	3,366,521

EXAMPLE 3

This example illustrates the relative effectiveness of three different embodiments of the reaction cell of the present invention: The Z-loop embodiment of Figure 6; the spiral embodiment of Figure 14; and the S-loop of Figure 2. 50 µl of magnetic microparticles bound to a chemiluminescent

substance are delivered from a fluidics handling apparatus to the respective detection cells. The magnetic microparticles are Nippon Paint 006 particles (Nippon Paint Co., Tokyo, Japan) of about 1.2 μm diameter and 50 percent magnetite by weight. They are bound to sulfopropyl acridinium and suspended in buffer at a concentration (by weight) of 0.15 percent solids. The trigger substance is an aqueous solution with 0.4% H_2O_2, 0.225 M NaOH, 2% Triton X-100, and 0.01% diethylene triamine pentaacetic acid. 50 μl of the magnetic microparticle suspension is transferred to a first conduit of each detection cell at a rate of about 4 ml/minute. Simultaneously, trigger is transferred into a separate conduit of the detection cell, whereupon the two separately sourced flows (acridinium labelled particles, and trigger solution) are merged at the entry point of the detection conduit of each cell. The trigger flow rate is maintained at that flow rate which results in maximum light emission, which flow rate was determined in advance for each different embodiment of the reaction cells. Light emission from the detection cell is measured by a Hamamatsu photomultiplier tube (Model R647-04). The results from the different reaction cells of the present invention are reported in Table 3, below.

TABLE 3

Reaction Cell Type	Z-Loop (Fig.6)	S-loop (Fig.2)	Spiral-loop (Fig 14)
Trigger Flow rate	8.0	6.0	4.0
Number of runs	10	15	4
Light Emission (relative light units)	614,921	1,497,360	440,122
Coefficient of Variation %	2.8	1.1	2.4
RLU ratio of reaction cell to conventional luminometer*	3.53	8.60	2.53

*The conventional luminometer noted in the above Table 3 involves high speed injection of trigger solution into a reaction test tube containing the chemiluminescent compound. To obtain the comparisons in this example the conventional luminometer used was a MGM luminometer Model Optocomp II. For the luminometer test, a single sample measurement was done as follows: 50 μl of the microparticle reagent is diluted with water to 400 μl and then

placed in a 12X 70 mm glass test tube. Then 300 µl of trigger solution is injected in to it at a high flow rate. Immediately afterward, photon emission is read for 5 seconds giving the relative light units as the total counts.

While particular embodiments and applications of the present invention have been illustrated and described, it is to be understood that the invention is not limited to the precise embodiments disclosed herein and that various modifications, changes, and variations which will be apparent to those skilled in the art may be made in the arrangement, operation, and details of construction of the invention disclosed herein without departing from the spirit and scope of the invention as defined in the appended claims. For example, the reaction cells schematically represented in Figures 18, 19 and 20 are illustrative of additional embodiments of the invention.

We claim:

1. A method for detecting a signal produced by a chemical reaction, said method comprising the steps of:

(1) providing a first flowable reactant in a first conduit and, separate therefrom, a second flowable reactant in a second conduit, said separate reactants being selected such that when contacted with one another, a detectable signal is produced;

(2) providing a continuous folded detection conduit which is transparent to said detectable signal and which is connected to said first and second reactant conduits, said detection conduit comprising two ends, and at one end thereof a first opening into which said first and second reactant conduits merge so as to be able to effectuate a merged flow from said first and second separate flowable reactants, and at another end thereof a second opening for discharge of said merged flow out of said detection conduit, whereby said reactants present in said first and second conduits can be caused to flow therefrom, into said detection conduit through said first opening, whereupon said merged flow can then be made to flow out of said detection conduit through said second opening; said detection conduit further comprising, between the first and second openings, at least one folded section, such folded section comprising, in continuous integral interconnection (i) a first segment of the detection conduit for chanelling the merged reactants along a first flow path; (ii) a second segment of the detection conduit for channelling the merged reactants along a second flow path, such that the first and second flow paths are channeled in different directions; and (iii) a third segment of the detection conduit constituting an elbow which joins the first and second segments; whereby said merged reactants can be channeled from said first flow path to said second flow path, and wherein said first, second and third segments together define a substantially non-circular path of travel for said merged reactants in said folded section;

(3) causing said first and second reactants to flow out of said first and second conduits and into said detection conduit whereupon said reactants are caused to merge with and contact one another such that said signal is produced in said detection conduit;

(4) providing means for detecting said signal produced in step (3), such means having a detection field and being positioned alongside the detection conduit, and wherein the detection conduit is folded to fit

substantially its entire length within said detection field of the signal detection means; and

(5) using the signal detection means of step 4 to detect the amount of signal produced in step 3 as a measure of the amount of reaction occuring between said merged reactants.

2. The method of claim 1 wherein said merged reactants include a suspension of microparticles labelled with a chemiluminescent compound, and said signal is in the form of light.

3. The method of claim 1 wherein said first fold segment and said second fold segment respectively define first and second flow paths which differ substantially from one another.

4. The method of claim 1 wherein said first fold segment and said second fold segment respectively define first and second flow paths which are essentially parallel.

5. The method of claim 4 wherein said folded detection conduit comprises a plurality of said folds, and said detection conduit is folded in an S-loop configuration.

6. The method of claim 4 wherein said folded detection conduit comprises a plurality of said folds, and said detection conduit is folded in a fingerprint configuration.

7. The method of claim 1 wherein said folded detection conduit comprises a plurality of fold sections, and wherein said detection conduit comprises a fold section disposed in a first plane, and another fold section disposed in a second plane substantially parallel to said first plane.

8. A method for detecting a signal produced by a chemical reaction, said method comprising the steps of:

(1) providing a first flowable reactant in a first conduit and, separate therefrom, a second flowable reactant in a second conduit, said separate reactants being selected such that when contacted with one another, a detectable signal is produced; and wherein at least one of said reactants

comprises a suspension of microparticles labelled with a signal producing compound.

(2) providing a spiral detection conduit which is transparent to said signal and which is connected to said first and second reactant conduits, said detection conduit comprising at one end thereof a first opening into which said first and second reactant conduits can merge to so as to be able to effectuate a merged flow from said first and second separate flowable reactants, and at another end thereof a second opening for discharge of said reactants out of said detection conduit, whereby said reactants present in said first and second conduits can be caused to flow therefrom, into said detection conduit through said first opening, whereupon said merged flow can flow out of said conduit through said second opening; said detection conduit being provided in the form of a continous spiral between said first and second opening;

(3) causing said first and second reactants to flow out of said first and second conduits and into said folded detection conduit whereupon said signal is produced upon contacting of said merged reactants;

(4) providing means for detecting the signal produced in step (3) and positioning said means adjacent said detection conduit, wherein said conduit fits substantially completely within a detection field of said signal detection means, and wherein said first opening of the detection conduit is within said detection field; and

(5) using said detection means to detect the amount of signal produced in step 3 as a measure of the amount of reaction occuring between said merged reactants.

9. The method of claim 8 wherein said first opening is positioned at the center of said spiral detection conduit.

10. The method of claim 8 wherein said signal is light energy produced by a chemiluminescent compound selected from the group consisting of acridinium sulfonamide compounds.

11. A reaction cell suitable for conducting a chemical reaction which produces a detectable signal, comprising: a housing and three separate conduits formed in said housing; wherein the conduits merge at a single point, and wherein one of the conduits is folded so as to comprise at least one folded section comprising, in continuous integral interconnection (i) a first segment of the conduit defining a first flow path; (ii) a second segment of said conduit

defining a second flow path differently directed from said first flow path; and (iii) a third segment of the folded conduit constituting an elbow joining said first and second segments, and wherein the first, second and third fold segments together define a non-circular flow path.

12. The reaction cell of claim 11 wherein said first and second flow paths differ substantially from one another.

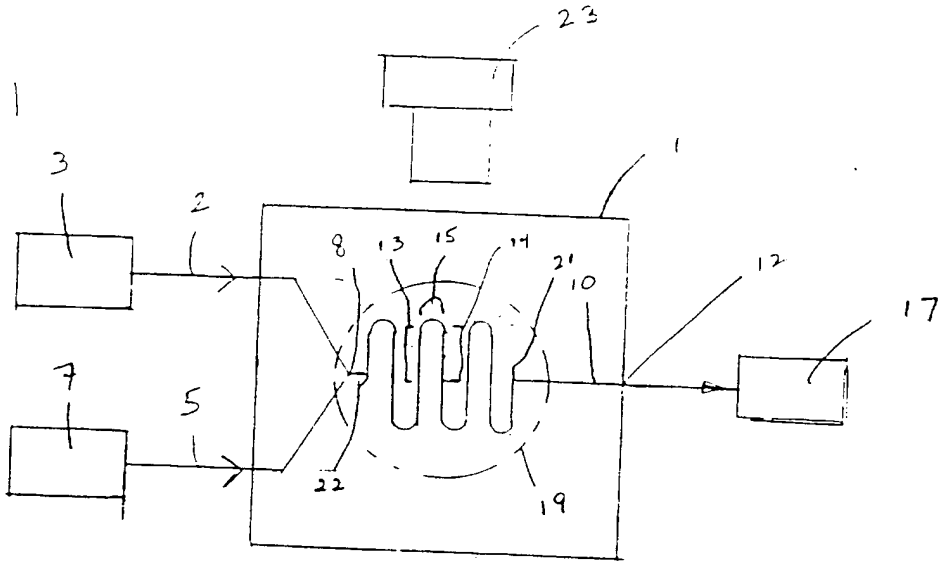
13. The reaction cell of claim 11 wherein said first and second flow paths are essentially parallel to one another.

14. The reaction cell of claim 13 wherein said folded detection conduit comprises a plurality of said fold sections, and said detection conduit is folded in an S-loop configuration.

15. The reaction cell of claim 13 wherein said detection conduit comprises a plurality of said fold sections, and said detection conduit is folded in a fingerprint configuration.

16. The reaction cell of claim 11 wherein said detection conduit comprises a plurality of folds, and wherein said detection conduit comprises at least one fold section disposed in a first plane, and at least one fold section disposed in a second plane substantially parallel to said first plane.

FIG. 1



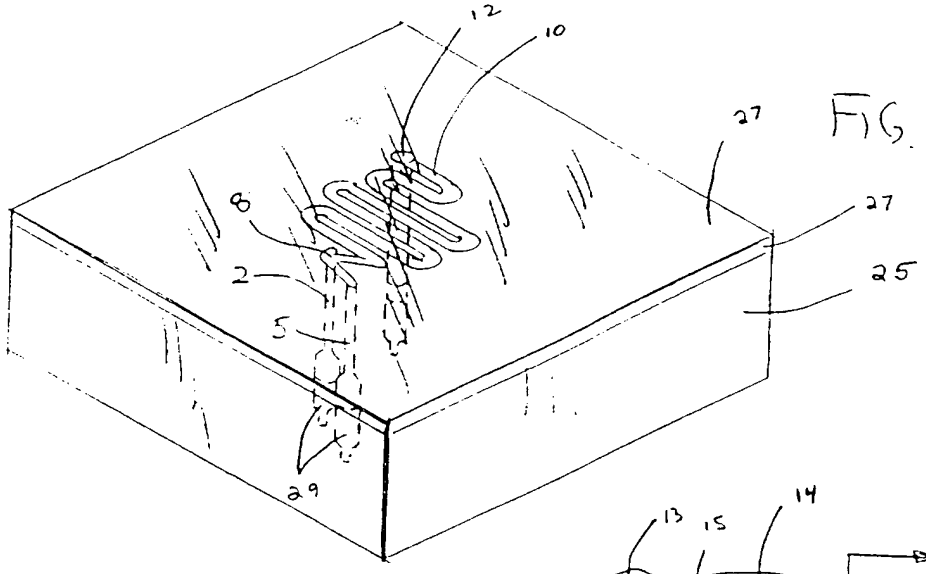
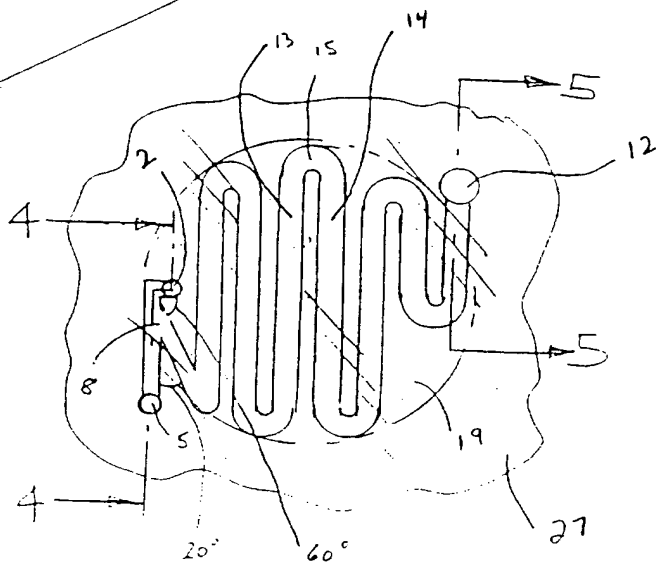


FIG. 2

FIG. 3



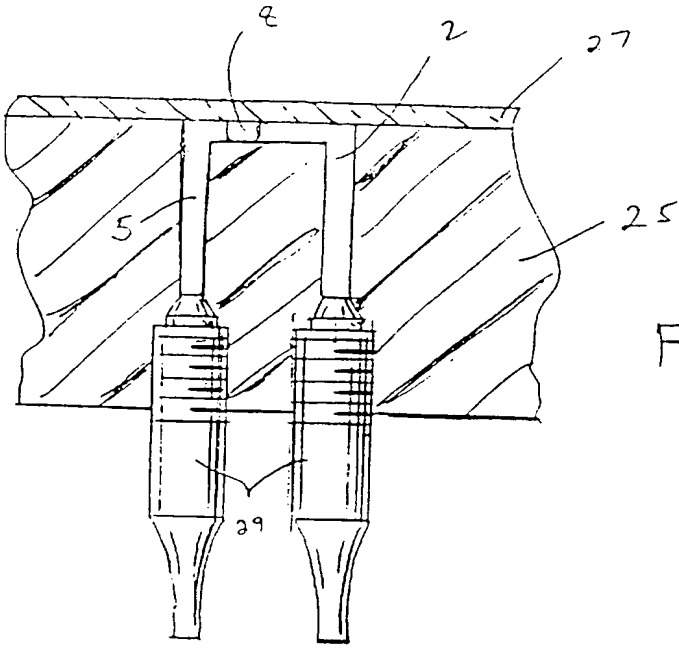


FIG. 4

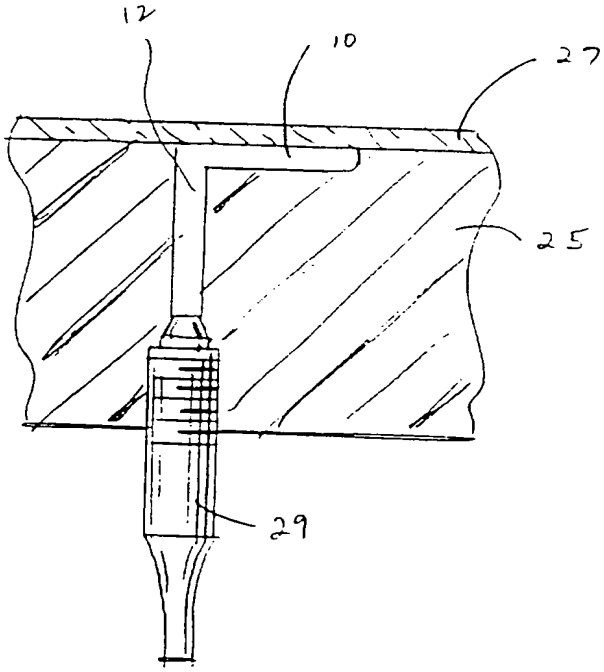


FIG. 5

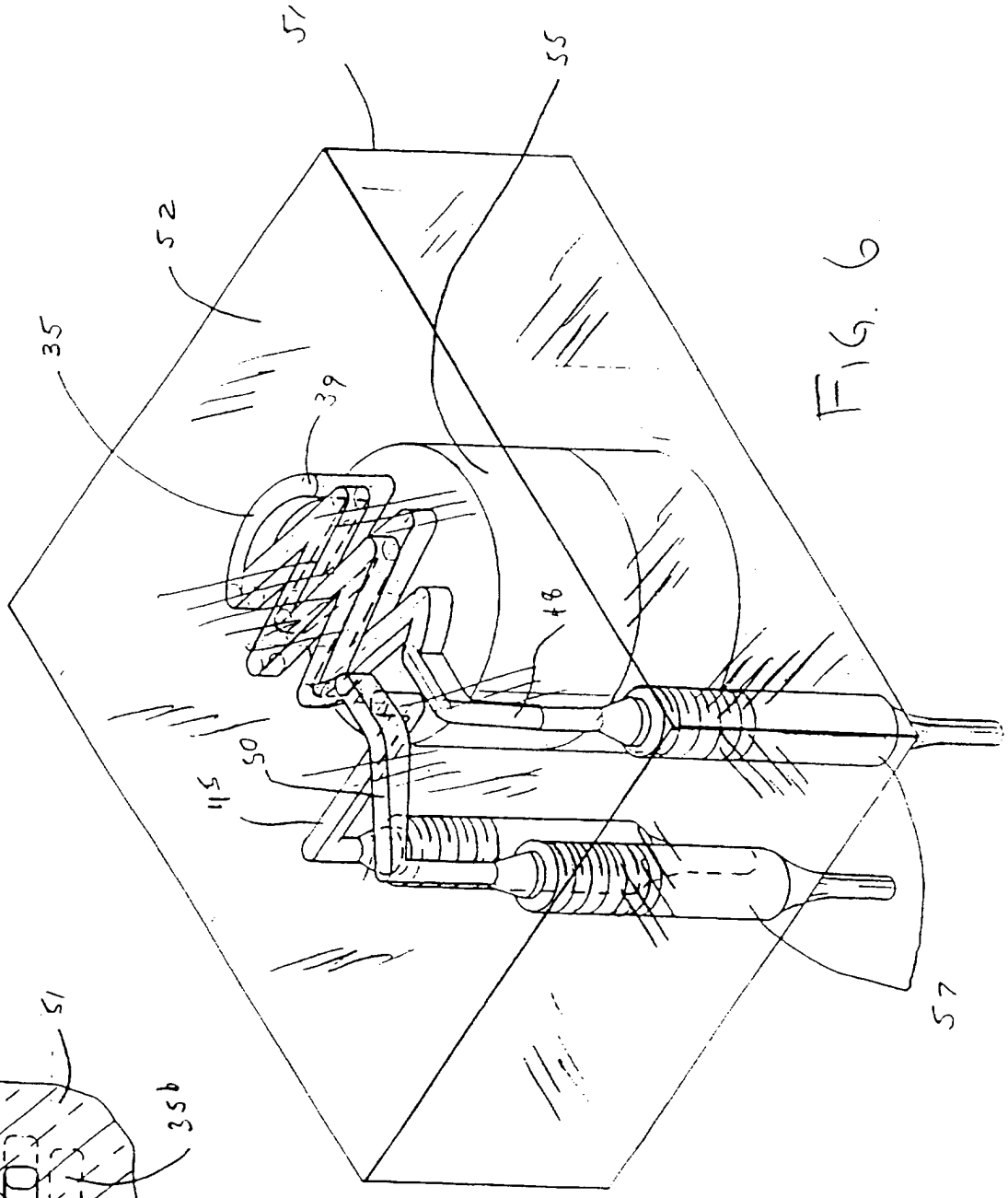


FIG. 6

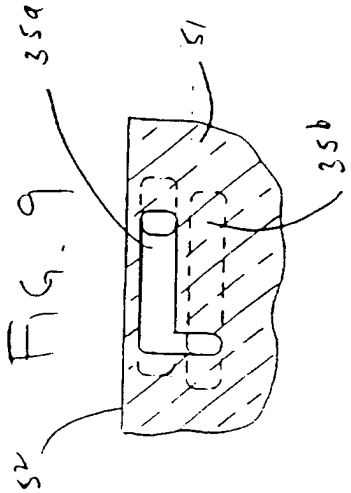
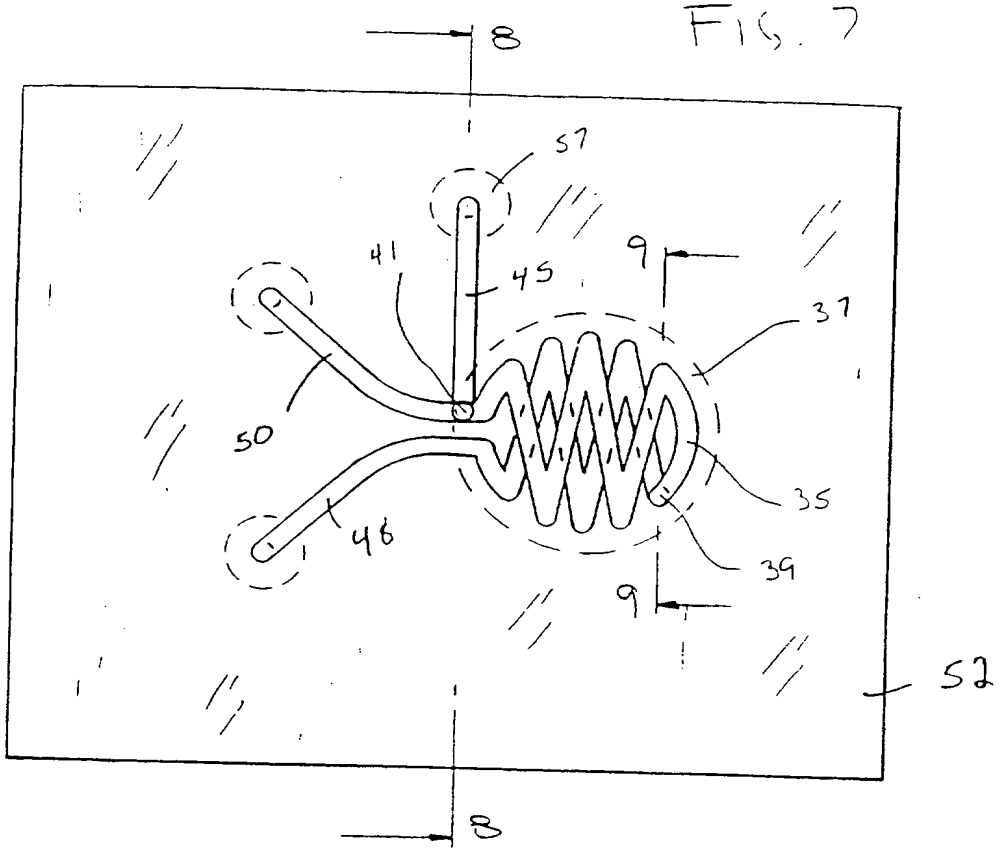


FIG. 9

FIG. 7



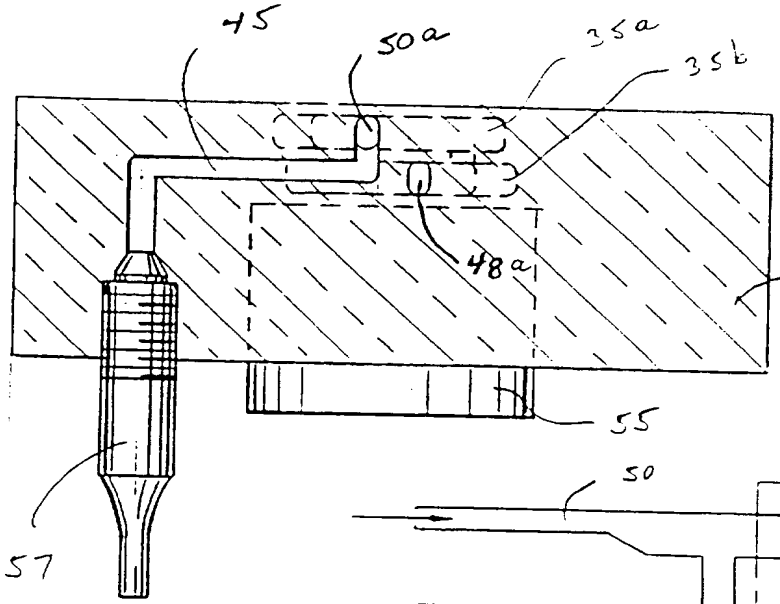


FIG 8

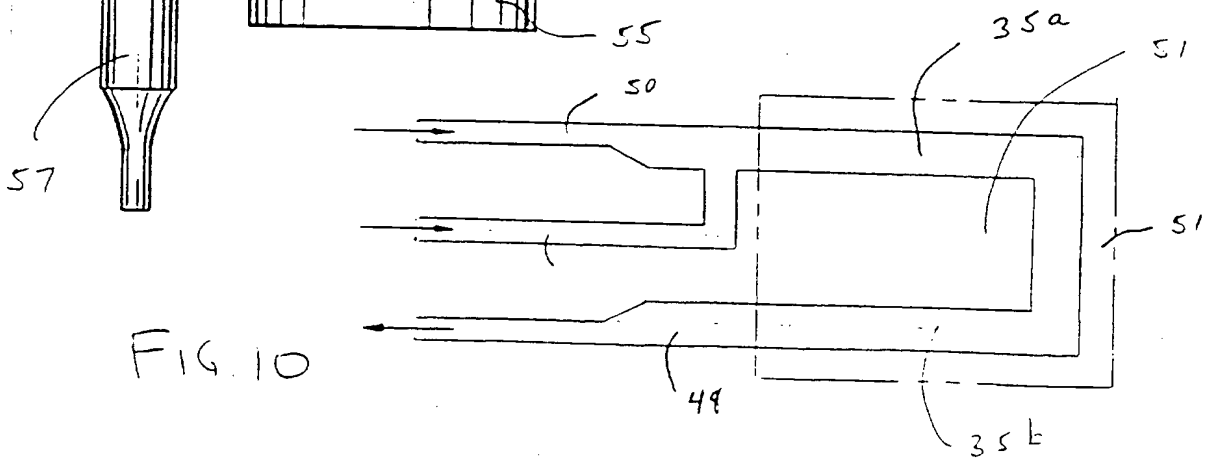


FIG. 10

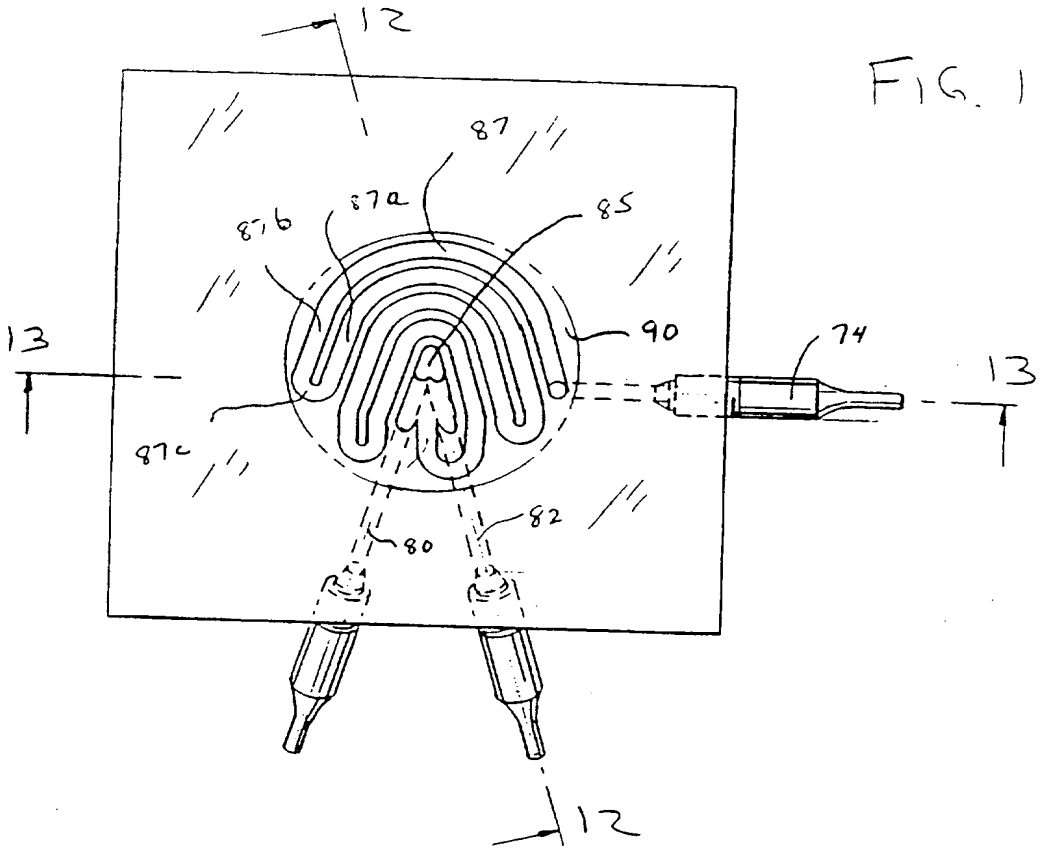


FIG. 11

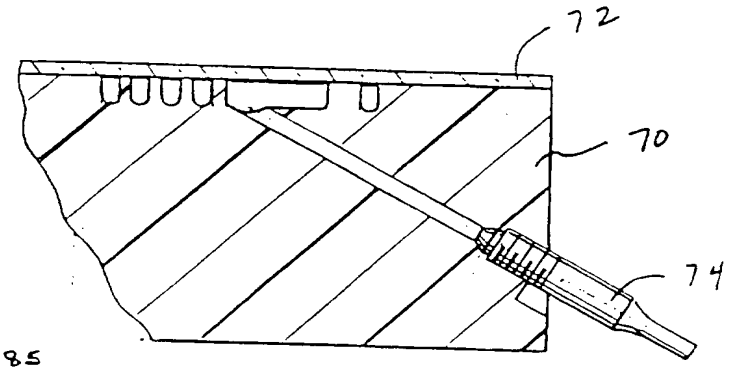


FIG. 12

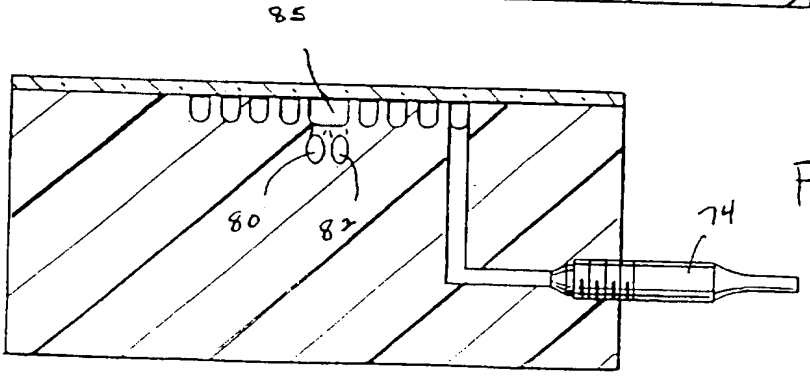


FIG. 13

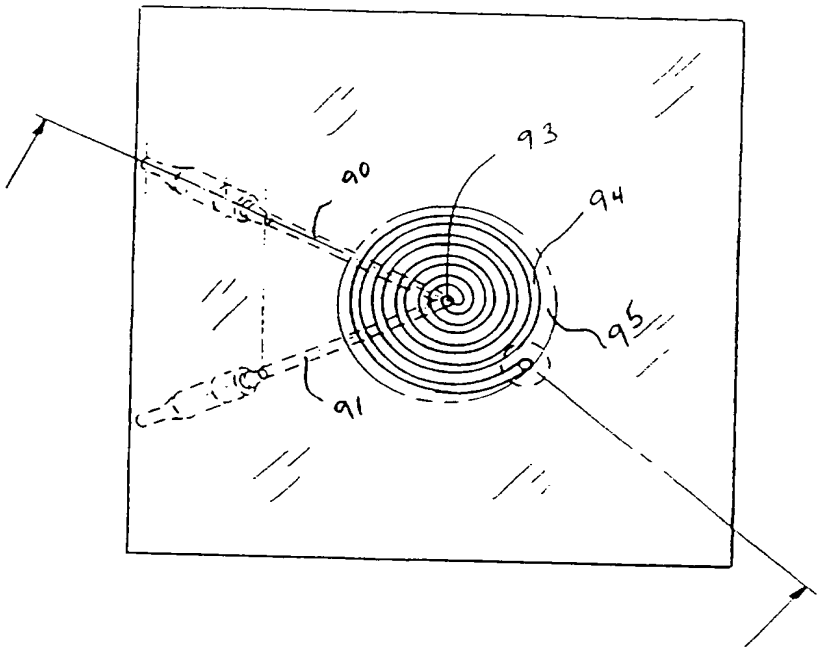


FIG. 14

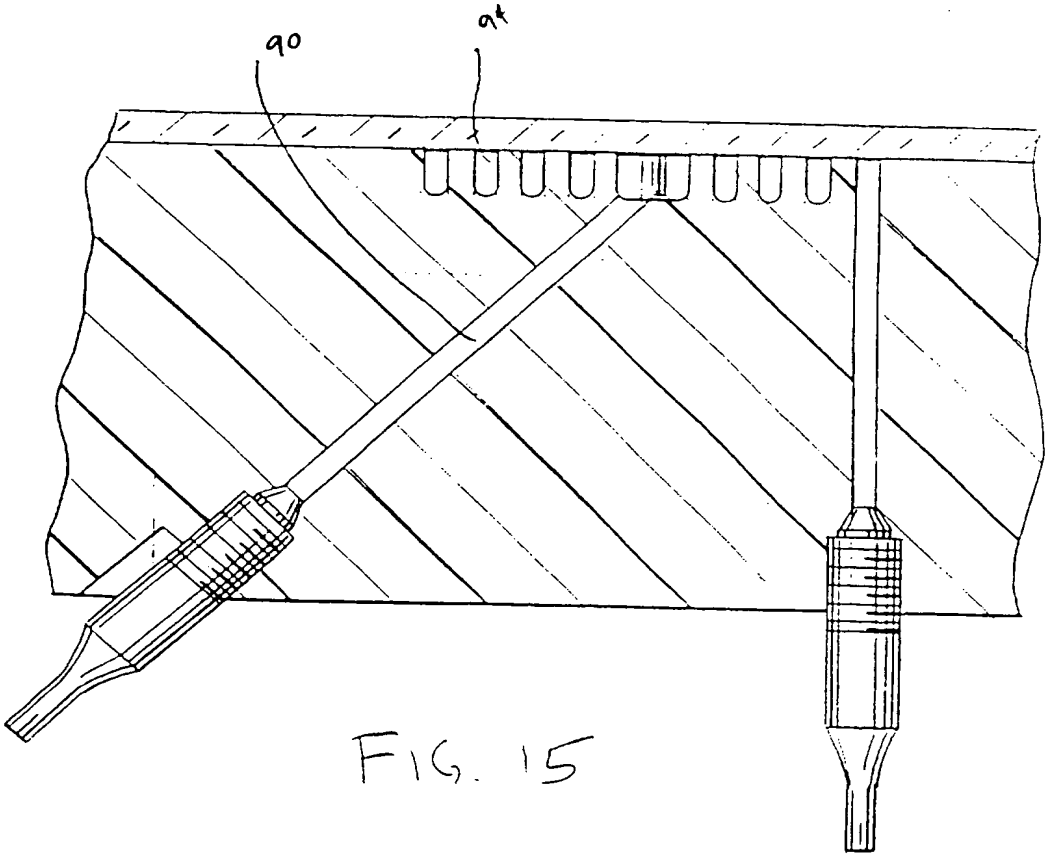


FIG. 15

FIG 16

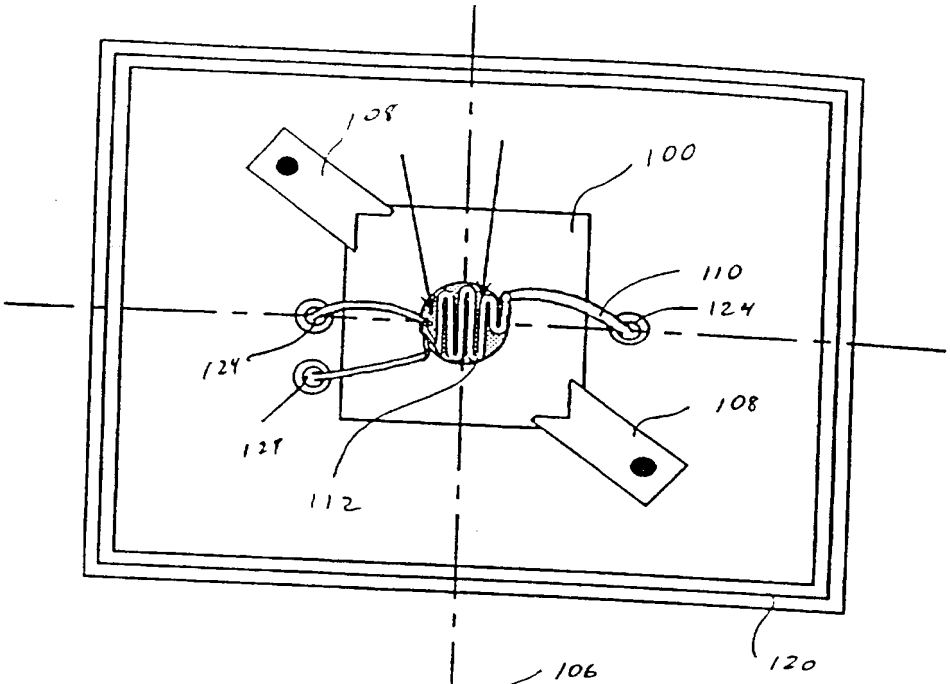
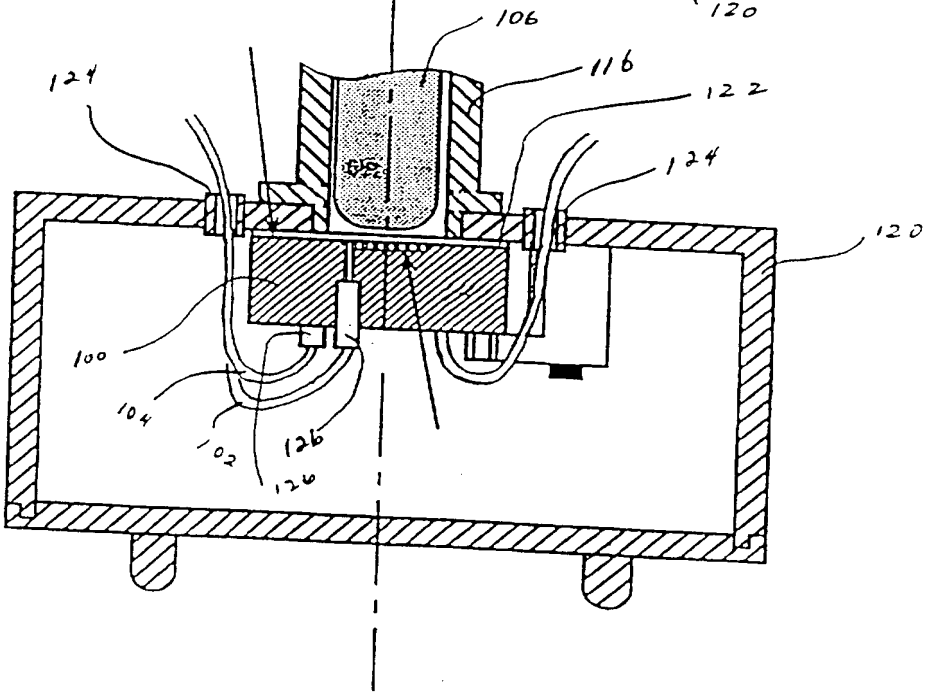


FIG 17



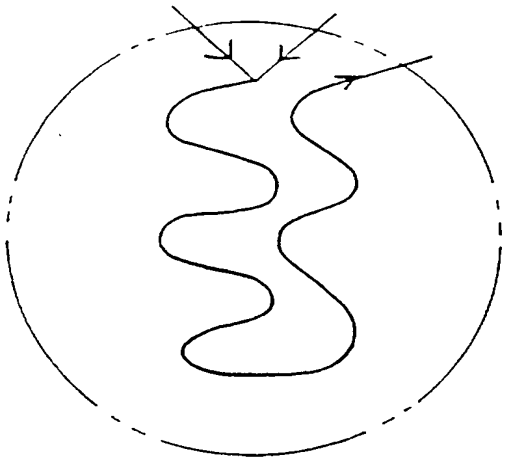


Fig. 18

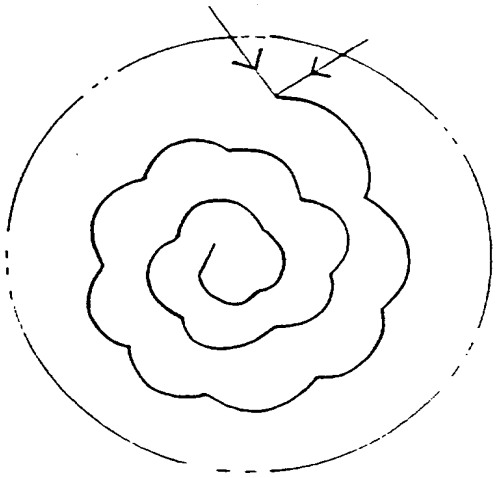


Fig. 19

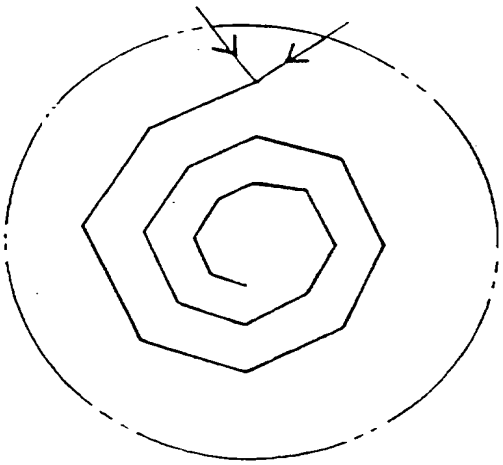


Fig. 20

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 96/02845

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/76 G01N21/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,3 679 312 (MANSBERG HYMAN P) 25 July 1972 see column 2, line 44 - column 3, line 36 see figures 1,2	8,9
Y		10
A		1,11

Y	EP,A,0 257 541 (HOECHST AG) 2 March 1988 cited in the application see claims 1,10,11,15	10

A	US,A,3 962 029 (WETTERMARK KARL GUSTAV GUNNAR ET AL) 8 June 1976 see column 4, line 61 - column 5, line 6 see figure 1	1,11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
3 July 1996	10.07.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Krametz, E

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 96/02845

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,5 250 259 (SUDA MASAYUKI) 5 October 1993 see column 2, line 12 - line 51 see figures 1,2 -----	1,11

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/US 96/02845

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		AU-B-	4249772	22-11-73
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		CA-A-	944584	02-04-74
		CH-A-	544938	15-01-74
		DE-A-	2228668	21-12-72
		FR-A-	2142353	26-01-73
		GB-A-	1363325	14-08-74
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		DE-D-	3751659	15-02-96
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		ES-T-	2083949	01-05-96
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		JP-B-	6099401	07-12-94
		JP-A-	63057572	12-03-88
		NO-B-	177639	17-07-95
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		DE-A-	2448731	30-04-75
		FR-A,B	2248504	16-05-75
		JP-C-	870822	20-07-77
		JP-A-	50065603	03-06-75
		JP-B-	51049004	24-12-76
		SE-A-	7314129	18-04-75
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